REMARKS

Reconsideration of the rejections set forth in the Office action mailed February 23, 2005 is respectfully requested. Claims 1-18 are currently under examination, and claims 35-36 are added by this amendment; claims 19-34 have been canceled.

I. Claim to Foreign Priority

Applicants acknowledge the requirement to file a certified copy of the priority document, and have ordered a copy of said document. The certified copy will be forwarded to the Examiner immediately upon its receipt. In accordance with 37 CFR §1.55(a)(2) and MPEP §201.13(II)(A), the certified copy will be filed before the grant of the patent.

II. Amendments

The Description of Figure 4 in the specification has been amended to remove the reference to Figure 4d.

SEQ ID NOs have been added at appropriate positions in the specification.

A new Sequence Listing has been filed for insertion at the end of the specification.

Claims 1 and 4 are amended to more clearly define the meaning of "immobilized", by reciting that "said 5' ends of both the nucleic acid template and the colony primers are immobilized to said solid support such that they cannot be removed by washing with water or aqueous buffer under DNA-denaturing conditions". Support is found, for example, at page 14, lines 19-27 of the specification.

New claims 35 and 36, dependent on claims 1 and 4, respectively, specify that the means of attachment for such immobilization is covalent attachment. Support is found, for example, at page 14, line 21 of the specification.

Claim 14 is amended to recite that the solid support referred to is the solid support to which said 5' ends of both the nucleic acid template and the colony primers are immobilized (in parent claim 1).

All of the dependent claims (2-3 and 5-18) are amended to change the formal language of the preamble.

No new matter is added by any of the amendments.

III. Objections to the Specification

As noted above, the description of Figure 4 in the specification has been amended to remove the reference to Figure 4d, and SEQ ID NOs have been added at appropriate positions in the specification. A new Sequence Listing has been filed incorporating all of the sequences in the specification having greater than 10 nucleotides.

IV. Objections to the Claims

As noted above, all of the dependent claims (2-3 and 5-18) are amended to change the formal language of the preamble (from "A method" to "The method"). The language objected to in claim 10 has been amended as suggested by the Examiner.

V. Rejections under 35 U.S.C. §112, Second Paragraph

Claims 14-15 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention.

The Examiner stated that it was unclear whether "said solid support" in claim 14 referred to "a solid support in a means for immobilizing the nucleic acid to a solid support in step (1) of claim 1 or...a solid support in a means for immobilizing the colony primer in step (1) of claim 1 or a solid support in step (2) of claim 1".

As noted above, claim 14 has been amended to recite that the solid support referred to is the solid support to which said 5' ends of both the nucleic acid template and the colony primers are immobilized (i.e., in step (2) of parent claim 1).

In view of the foregoing, the applicants submit that amended claims 14-15 comply with the requirements of 35 U.S.C. §112, second paragraph.

VI. Rejections under 35 U.S.C. §102(b)

Claims 1-3, 10-12, 14 and 15 were rejected under 35 U.S.C. §102(b) as being anticipated by Adams *et al.*, WO 96/04404. This rejection is respectfully traversed for the following reasons.

A. The Invention

The applicant's invention, as embodied in claim 1, is directed to a method for amplification of at least one nucleic acid, comprising the following steps:

(1) forming at least one nucleic acid template comprising the nucleic acid to be amplified, wherein the nucleic acid contains an oligonucleotide sequence Y at the 5' end and an oligonucleotide sequence Z at the 3' end, and the nucleic acid carries a means for immobilizing the nucleic acid to a solid support at the 5' end;

(2) mixing the at least one nucleic acid template, in the presence of a solid support, with one or more colony primers X, each of which can hybridize to the oligonucleotide sequence Z and carries a means for immobilizing the colony primer to a solid support at the 5' end, whereby the 5' ends of both the nucleic acid template and the colony primers are immobilized to the solid support;

wherein said 5' ends of both the nucleic acid template and the colony primers are immobilized to said solid support such that they cannot be removed by washing with water or aqueous buffer under DNA-denaturing conditions; and

(3) performing one or more nucleic acid amplification reactions on the immobilized nucleic acid template, so that nucleic acid colonies are generated.

With reference to immobilization of the nucleic acid template and colony primers to the solid support (step (2) of the claim), claims 1 and 4 as amended specifically recite that these molecules are immobilized to the support "such that they cannot be removed by washing with water or aqueous buffer under DNA-denaturing conditions". See, for example, the specification at page 14, lines 19-27.

B. The Prior Art

The cited reference, Adams *et al.*, does not teach step (2) of applicants' claimed method, in which colony primers are mixed with nucleic acid templates in the presence of a solid support, "whereby the 5' ends of both the nucleic acid template and the colony primers are immobilized to the solid support", where "immobilized" is defined as stated in the claims.

In particular, Adams does not show a method in which nucleic acid templates are "immobilized to the solid support" (step (2) of applicants' claim) prior to the step of

"performing one or more nucleic acid amplification reactions" on the nucleic acid templates (step (3) of applicants' claim).

As shown in Figures 1 and 2 of Adams, for example, it is clear that, prior to any amplification reaction, nucleic acid templates are bound only by hybridization to the primer. A nucleic acid template that is bound only by hybridization would, by definition, "be removed...under DNA-denaturing conditions".

It is clear from the description, as set forth below, that the "nucleic acid template" in Adams is removed from the support under "DNA-denaturing conditions". (Note that in Adams, the nucleic acid template, *i.e.* the target sequence to be amplified, is referred to as the "first nucleic acid", and the immobilized primers are referred to as "second" and/or "third" nucleic acids. See *e.g.* page 14, lines 5-15 and 29-30.)

For example, with respect to Figure 1D, the Adams reference states that "FIG. 1D depicts *an annealed, or hybridization product* comprising first nucleic acid strand 25" (page 15, lines 9-11; emphasis added). Subsequently, "Upon imposition of denaturation conditions, *a denaturation product is formed* comprising first nucleic acid strands 25 and 27" (page 16, lines 6-8; Fig. 1F; emphasis added). Fig. 1F clearly shows that strand 25 is no longer attached to the solid support.

With respect to Fig. 2C, the Adams reference states that "an *annealed product* is formed in area 121 comprising a first nucleic acid 133 and a second nucleic acid 125" (page 19, lines 6-8; emphasis added). Subsequently, "the fifth work station imposes *denaturation conditions* on the elongation product 145 *to allow first nucleic acid strand 133 to dissociate* from second nucleic acid 125" (page 19, lines 33-35; emphasis added).

Because the nucleic acid template in Adams "dissociates" from the primer under denaturing conditions, it cannot be considered "immobilized" as defined in the applicants' claims. Thus, Adams *et al.* does not teach immobilization of the template nucleic acid to the solid support prior to amplification, as claimed by the applicants.

Since the reference does not disclose all of the elements set out above in independent claim 1, this claims and its dependent claims cannot be anticipated by this reference under 35 U.S.C. §102(b). In view of this, the applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. §102(b).

VII. Rejections under 35 U.S.C. §102(e)

Claims 1-3, 10-12, 14, 15 and 17 were rejected under 35 U.S.C. §102(e) as being anticipated by Adams *et al.*, U.S. Patent No. 6,060,288. This rejection is respectfully traversed for the following reasons.

The disclosure of this U.S. patent is very similar to that of the Adams *et al.* PCT publication discussed above, with which it shares priority, with the exception of new Examples 5-8 in the U.S. patent. Applicants note that the Examiner's characterization of the U.S. patent (pages 9-13 of Office Action) is essentially identical to the Examiner's characterization of the PCT publication (pages 5-9 of Office Action), with the exception of cited column and line numbers, and the reference to applicants' dependent claim 17.

With respect to the invention of independent claim 1, there is no substantial difference between the disclosures of these two references. Therefore, for the same reasons discussed above, Adams *et al.* does not teach step (2) of applicants' claimed method.

Since the reference does not disclose all of the elements set out above in independent claim 1 and its dependent claims, these claims cannot be anticipated by this reference under 35 U.S.C. §102(e). In view of this, the applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. §102(e).

VIII. Rejections under 35 U.S.C. §103(a)

Claim 4 was rejected under 35 U.S.C. §103(a) as being unpatentable over Adams *et al.*, 1996 (above) in view of Huang, U.S. Patent No. 5,645,994.

Claims 5, 6, 8, and 9 were rejected under 35 U.S.C. §103(a) as being unpatentable over Adams *et al.*, 1996 (above) in view of Bukh *et al.*, U.S. Patent No. 5,514,539.

Claim 7 was rejected under 35 U.S.C. §103(a) as being unpatentable over Adams *et al.*, 1996 (above) in view of Bukh *et al.* (above) and further in view of Hildebrand *et al.*, U.S. Patent No. 6,287,764.

Claim 13 was rejected under 35 U.S.C. §103(a) as being unpatentable over Adams *et al.*, 1996 (above) in view of Lund *et al.*, *Nucleic Acids Research* 16:10861-80 (1988).

Claims 16 and 18 were rejected under 35 U.S.C. §103(a) as being unpatentable over Adams *et al.*, 1996 (above) in view of Fodor *et al.*, U.S. Patent No. 5,800,992.

The rejections are respectfully traversed in light of the following remarks.

A. The Invention

The invention of independent claims 1 and 4, as described above, includes the steps of:

"(2) mixing...at least one nucleic acid template, in the presence of a solid support, with one or more [degenerate] colony primers...whereby the 5' ends of both the nucleic acid template and the colony primers are immobilized to the solid support

wherein said 5' ends of both the nucleic acid template and the colony primers are immobilized to said solid support via a means of attachment selected from covalent attachment, irreversible passive adsorption, and affinity between molecules, such that said attachment cannot be removed by washing with water or aqueous buffer under DNA-denaturing conditions; and

(3) performing one or more nucleic acid amplification reactions on the immobilized nucleic acid template, so that nucleic acid colonies are generated".

Accordingly, the invention is directed to an amplification method in which, prior to amplification, the nucleic acid template is immobilized, as defined in the claim, to a solid support. As discussed above, in Section VI of this response, such a method is not taught in the primary cited reference, Adams *et al.*

As discussed in the previous response, the applicants' specification describes the beneficial effects that can be achieved via this "initial immobilization" of the template, in contrast to prior art methods (including the primary cited reference, Adams *et al.*, W096/04404) in which the template is not immobilized, and can be removed under DNA-denaturing conditions:

> The initial immobilisation of the template nucleic acid means that the template nucleic acid can only hybridise with colony primers located at a distance within the total length of the template nucleic acid. Thus the boundary of the nucleic acid colony formed is limited to a relatively local area to the area in which the initial template nucleic acid was immobilised. (page 23, line 35 to page 24, line 4)

It can thus be seen that the method of the present invention allows the generation of a nucleic acid colony from a single immobilised nucleic acid template

and that the size of these colonies can be controlled by altering the number of rounds of amplification that the nucleic acid template is subjected to. Thus the number of nucleic acid colonies formed on the surface of the solid support is dependent upon the number of nucleic acid templates which are initially immobilised to the support....

Such so called "autopatterning" of nucleic acid colonies has an advantage over many methods of the prior art in that a higher density of nucleic acid colonies can be obtained due to the fact that the density can be controlled by regulating the density at which the nucleic acid templates are originally immobilised. Such a method is thus not limited by, for example, having specifically to array specific primers on particular local areas of the support and then initiate colony formation by spotting a particular sample containing nucleic acid template on the same local area of primer. The numbers of colonies that can be arrayed using prior art methods, for example those disclosed in W096/04404 (Mosaic Technologies, Inc.) is thus limited by the density/spacing at which the specific primer areas can be arrayed in the initial step (page 26, line 21 to page 27, line 17).

B. The Cited Art

As established in Section IV of these Remarks, neither the primary cited reference, Adams *et al.*, 1996 (WO 96/04404), nor its U.S. equivalent, U.S. Patent No. 6,060,288, teaches the method of independent claim 1.

Nor does Adams *et al.* provide any motivation to alter the disclosed method along the lines of the applicants' invention, by immobilizing the nucleic acid template to the solid support, such that it cannot be removed under DNA-denaturing conditions. The method of Adams *et al.* is described as useful for "the detection of the presence of (or the absence of) a target nucleic acid sequence in a test sample" (column 1, lines 50-54 of US patent). In preferred embodiments, the method "further comprises the step of monitoring the support for the presence of one or more amplification products, in which one or more amplification products are indicative of the presence of one or more target sequences, and in which *absence* of an amplification product is indicative of the *absence* of a target sequence" (page

6, lines 30-34 of PCT; claim 2 of PCT; sentence bridging columns 3-4 of US patent; emphasis added).

Because the method of Adams *et al.* is intended to indicate when a target sequence is absent from a sample, there would clearly be no reason to immobilize the target sequence to the support, such that it cannot be removed under DNA-denaturing conditions, since this would produce a false positive result, indicating that the target sequence was present.

The secondary references were cited for their disclosure of various individual features not recited in independent claim 1. The applicants refer to the response filed October 19, 2004 for brief discussion of the teachings of these references, which are also reproduced below.

None of these references make up for the deficiencies of the primary reference(s) with respect to the applicants' independent claims. That is, none of them discloses or suggests an amplification method in which a nucleic acid template and primers are immobilized to a solid support prior to amplification, as defined in the claims. Therefore, the combination of any or all of these references with Adams *et al.* does not disclose or suggest the claimed amplification method.

Huang, which is concerned with identification of disease-causing microbes in biological samples (as stated in the Field of the Invention), is cited for the disclosure of "universal primers".

The applicants' specification defines a "degenerate primer", as recited in dependent claim 4, as follows: "'Degenerate primer sequences' as used herein refers to a short oligonucleotide sequence which is capable of hybridizing to any nucleic acid fragment independent of the sequence of said nucleic acid fragment" (page 13, lines 31-34).

The "universal primers" in Huang are designed based on a protein which is ubiquitous in species desired to be detected in a sample (*e.g.* bacterial or microbial species) and which has both highly conserved and variable sequences. Preferably, the protein is type II topoisomerase, and the "universal primers" are based on highly conserved regions of this protein (column 3, line 66 to column 4, line 29). The "universal primers" described in Huang would not be "capable of hybridizing to any nucleic acid fragment independent of the

sequence of said nucleic acid fragment".

As stated above, moreover, even if Huang did teach "degenerate primers", its combination with the primary reference(s) would not teach the applicants' invention.

Bukh *et al.*, which is directed to identification and uses of envelope 1 gene sequences of HCV isolates, is cited for the disclosure of sequencing of PCR products and the use of labeled primers for detection of the amplification products. A conventional method of sequencing (dideoxy-nucleotide chain termination method) is used in Example 1 of Bukh *et al.* However, sequencing by to incorporation and detection of labeled nucleotides (as recited in applicants' claim 6) is not described in Bukh *et al.*

Adams *et al.*, the primary reference, is further cited for disclosure of the visualization of nucleic acid colonies, which may employ a labeled or unlabeled nucleic acid probe.

Hildebrand *et al.*, which is directed to methods of HLA Class I typing by sequencing, is cited for its disclosure of simultaneous sequencing of PCR products. In the passage pointed out by the Examiner, ten PCR products are sequenced simultaneously using the "AutoLoad Solid Phase Sequencing Kit". Because the PCR products must be bound to "combs" provided with the kit (column 12, lines 37-43), this procedure would be less than ideal for sequencing PCR products which are already bound to a solid support.

Lund *et al.*, which is directed to a study of methods of attaching nucleic acids to magnetic beads, is cited for its disclosure of the use of a 5'-amino group for attachment of a nucleic acid to carboxyl-functionalized magnetic beads.

Fodor *et al.*, which is directed to the use of large arrays of nucleic acids for sequencing, is cited for its disclosure of supports having attached nucleic acids at a density of 10,000-100,000/mm^2. Such supports are prepared by use of "masking technology and photosensitive synthetic subunits (column 7, lines 55-56).

The teachings of these references, directed to various technologies, provide no guidance regarding a method in which a nucleic acid template and primers are immobilized to a solid support, such that it cannot be removed under DNA-denaturing conditions, followed by amplification of the template. No combination of the teachings of the secondary references

with those of Adams *et al.* would have suggested the claimed method, or the benefits thereof, to one skilled in the art.

In view of the foregoing, the applicants respectfully request the Examiner to withdraw the rejections under 35 U.S.C. §103(a).

IX. Conclusion

In view of the foregoing, the applicant submits that the claims now pending are now in condition for allowance. A Notice of Allowance is, therefore, respectfully requested.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 838-4403.

Respectfully submitted,

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